Immunology 1987 60 261–268

Modulation of human monocyte Fc receptor function by surface-adsorbed IgG

T. W. JUNGI,* G. VON BELOW,* P. G. LERCH† & P. J. SPAETH† **Institute for Clinical and Experimental Cancer Research, University of Berne, and* †*Central Laboratory, Swiss Red Cross Blood Transfusion Service, Berne, Switzerland*

Accepted for publication 6 October 1986

SUMMARY

Fc receptor-mediated phagocytosis was measured with monocytes subjected to various treatments. Monocytes exposed to IgG during their adherence, or after they had adhered to a surface, experienced functional impairment. This was manifested in the requirement of a higher antibody density on target particle for efficient phagocytosis, and in an enhanced susceptibility to inhibition by fluid-phase IgG. The impairment was found to be due to an interaction of IgG adhering to the surface with the Fc receptors. This effect could be induced with monomeric IgG, devoid of IgG aggregates or immune complexes. IgG coatings that resulted in inefficient Clq fixation promoted considerable functional impairment of monocytes within 1 hr. In addition, the prolonged contact of monocytes with polystyrene in the absence of IgG also led to a functional reduction. The study points to a compromised function of phagocytes exposed to artificial surfaces.

INTRODUCTION

The binding of immunoglobulin-covered particles by phagocyte receptors for the Fc portion of immunoglobulin G (IgG) is regarded as an important step preceding their endocytosis and the activation of microbicidal mechanisms (Silverstein, Steinman & Cohn, 1977). Efficient phagocytosis depends on the expression of Fc receptors (FcR), on a functioning system transducing the signal to the motile apparatus, and on an undisturbed function of the motile apparatus itself. We have recently shown that human monocytes differentiating *in vitro* to macrophages undergo a state of anergy with respect to FcR-mediated phagocytosis (Jungi & Hafner, 1986). The failure to ingest suboptimally opsonized particles and the exquisite sensitivity to inhibition by fluid-phase IgG are correlated with a reduction in the number of surface-expressed FcR. This receptor modulation is reminiscent of the loss of functional FcR following exposure to IgG aggregates and immune complexes (Kurlander, 1980; Michl, Pieczonka, Unkeless & Silverstein, 1979; Michl *et al.*, 1983; Ragsdale & Arend, 1980). However, the condition under which monocytes were cultured *in vitro* made a participation of aggregated IgG or immune complexes unlikely. In the present report, the hypothesis was tested as to whether monomeric IgG adsorbed out of the medium to the cell culture vessel is able to modulate FcR function. The findings illustrate that media containing monomeric IgG and non-IgG proteins in physiological proportion cover artificial surfaces in a manner suited for compromising phagocyte functions.

Correspondence: Dr T. W. Jungi, Institute for Clinical and Experimental Cancer Research, University of Berne, Tiefenau Hospital, CH-3004 Berne, Switzerland.

MATERIALS AND METHODS

Chemicals

The following preparations of IgG were used: standard gammaglobulin 16% (IgG-IM; SRC Blood Transfusion Service, Berne, Switzerland), deprived of the majority of aggregates by ultracentrifugation (Jungi & Barandun, 1985); intact human IgG subjected to acid (pH 4) in order to achieve i.v. tolerance (IgG-IV; SRC Blood Transfusion Service); the monomer-enriched fraction of ion exchange chromatography-purified IgG from pooled human serum, prepared as described elsewhere (Jungi *et al.*, 1986a); the monomer fraction of a similarly purified IgG1 myeloma protein (Hug); lyophilized rabbit and bovine IgG (Miles Scientific, purchased through Dr Streuli AG, Zurich, Switzerland); human IgG deprived of the Fc portion by pepsin treatment (Gammavenin; Behringwerke, Marburg, FRG); and human IgG treated by limited sulfitolysis (Venimmun; Behringwerke). Lyophilized IgG preparations were dissolved in medium (see below) immediately before use; the others were stored at $-80°$ in small aliquots. Human IgG preparations were analysed by high-performance size exclusion chromatography for the content of IgG split products or high molecular weight aggregates as described previously (Jungi *et al.*, 1986a).

The medium used was Dulbecco's minimum essential medium (MEM; Seromed, Munich, FRG) buffered with HEPES (0·025 M) and supplemented either with 2% (v/v) fetal calf serum (FCS; Seromed) or with 0·5% (w/v) albumin. The latter was bovine serum albumin (BSA; Boseral S; Organon Technika, Turnhout, Belgium) and in some experiments human serum albumin (HSA; Behringwerke).

Preparation of monocyte monolayers
Mononuclear cells were isolated by a slightly modified Ficoll–Triosyl technique (Bøyum, 1968) as described elsewhere (Jungi & Barandun, 1985). They were resuspended at $2{\cdot}5\times10^6$ to 3×10^6 per ml in the above medium and dispensed in 100-μl aliquots in flat-bottomed microtitre plate wells (Nunc, Roskilde, Denmark). Depending on the experimental protocol, IgG was included in the medium, or FCS was replaced by human serum. Following an incubation of 60 min in a humidified chamber kept at 37°, medium and non-adherent cells were removed by rinsing, and adherent monocytes forming a monolayer were kept for another 30 min in fresh, IgG-free medium before the phagocytosis test was started.

Opsonization of sheep erythrocytes
Opsonization of sheep erythrocytes (E) was performed by classical methods (Kabat & Mayer, 1961) using affinity-purified rabbit anti-E IgG antibodies (Cordis, Miami, FL). The number of antibodies per E was determined radiometrically by means of ^{125}I-labelled anti-E IgG (Jungi & Barandun, 1985).

Spectrometric phagocytosis test
Phagocytosis was assayed spectrometrically, as recently described (Jungi, 1985). In brief, sheep erythrocytes (E) opsonized with varying known amounts of anti-E antibodies were offered to monolayer cells. After an ingestion phase (60 min at 37°), non-ingested E were removed by washing and by hypotonic lysis. Monolayers were then lysed with sodium dodecyl sulphate (0·1% w/v), and a mixture of H_2O_2 (35 mM) and tetramethylbenzidine (4 mM) was added to each well. The extent of substrate conversion assayed in an automated ELISA reader (AM 130, Dynatech, Billinghurst, Sussex, U.K.) at 405 nm was proportional to the amount of haemoglobin released from ingested E (Jungi & Hafner, 1986). A calibration curve, established with monolayers to which known numbers of E were added, permitted the quantification of ingested E per well, and this was related to the number of phagocytes per monolayer. The latter was determined microscopically as described elsewhere (Nakagawara & Nathan, 1983). Phagocytic indices (ingested E per monocyte) were quantified for each of eight E probes, representing different densities of opsonizing antibodies on their surface.

Phagocytosis inhibition test
Monolayer cells were assayed for their susceptibility to inhibition of phagocytosis by IgG. In these assays, optimally sensitized E were coincubated with varying amounts of IgG-IM in the fluid phase, and ID_{50} (dose at which IgG inhibited phagocytosis by 50 %) was determined, using log-linear regression analysis as described previously (Jungi & Hafner, 1986).

Cell culture
Mononuclear cells were cultured for 18 hr either in tissue culture flasks (Falcon, Oxnard, MD) or in teflon bags (FEP 100 A, Dupont de Nemours, Geneva, Switzerland). Cells from flasks were removed by vigorous shaking of chilled flasks on a vortex mixer; cells from hydrophobic containers were collected after gentle rubbing of the bags. After washing cells twice in phosphate-buffered (0·1 M, pH 7·4) saline, they were resuspended in medium and allowed to adhere to microtitre plate wells. The medium was supplemented either with 10% (v/v) FCS and IgG or with FCS alone.

Mononuclear cells were also used for short-term culture (1 hr) in Nunc Minisorp tubes, which were agitated in order to prevent the interaction of cells with tube surfaces. These cells were washed and plated as described above. Medium contained either IgG and albumin (0·5% w/v) or albumin alone.

Radiometric binding tests
Monomeric IgG and IgG-IV were labelled by the chloramine T method (Sonoda & Schlamowitz, 1970) and dialysed for 2 days against PBS containing 0·003 M NaN_3. Radioactivity was contained to >90% in the trichloroacetic acid precipitable fraction, and the specific activity was between 3000 and 5000 c.p.m./ng IgG. Binding tests were performed with mixtures of labelled and unlabelled IgG in wells (Nunc) which could be counted individually in a gammacounter after vigorous washing.

Clq as purified by affinity chromatography according to Kolb, Kolb & Podack (1979). It was labelled with ^{125}I by the lactoperoxidase method as described by Spaeth *et al.* (1983). This material was allowed to bind to microtitre plate wells precoated with monomeric IgG or aggregated IgG, respectively, in a 2-hr incubation at 37° in veronal buffer (Kabat & Mayer, 1961) containing albumin (0·5%).

Data transformation and statistical analysis
All phagocytosis determinations were performed in triplicate. In some experiments, linear regression analysis was applied to the log-linear portion of the sigmoid dose–response curves of phagocytosis tests, and the antibody density on E resulting in a half maximal phagocytic index was calculated. This value was larger for cells exposed to IgG than for counterparts that had not been exposed to IgG, and the logarithmic difference was taken as a measure for the degree of Fc receptor modulation (Fig. 5a)

Binding tests were run in quadruplicate to octuplicate, and comparable groups were subjected to a *t*-test analysis for significance.

RESULTS

Influence of IgG on the Fc receptor-mediated phagocytosis by polystyrene-adherent monocytes

Monocytes that were allowed to adhere to polystyrene microtitre plate wells for 60 min were tested for their capacity to ingest erythrocytes sensitized with varying known amounts of rabbit anti-E antibodies. Monocytes preincubated in medium containing either serum IgG or purified IgG-IV during the adherence phase, then washed and incubated for another 30 min in the absence of IgG, showed a reduced capacity to recognize E opsonized with low antibody numbers, as evidenced by a shift of the dose–response curve to the left (Fig. 1a). They also displayed an enhanced susceptibility to inhibitor IgG in solution (Fig. 1b). In the following, this functional impairment is referred to as modulation of FcR-dependent phagocytosis. In subsequent experiments, monocytes were allowed to adhere to polystyrene and then cultured overnight. Thereafter, they were detached from the surface, washed and placed in microtitre plates for the phagocytosis test. Modulation of FcR-dependent phagocytosis occurred regardless of whether IgG was present during the adherence phase only, or whether it was given to monocytes in

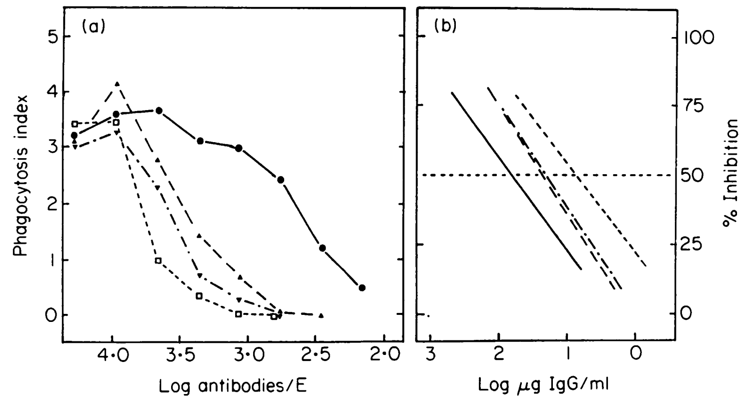

Figure 1. Influence of IgG and human serum on the Fc receptor-mediated phagocytosis by monocytes. Monocytes were allowed to adhere to polystyrene in medium containing either 2% (v/v) FCS (●——●), or FCS and 0·25 mg/ml IgG-IV (▲– –▲), or 2% (v/v) fresh human serum (▼–·–▼), or 2% heat-inactivated (30 min 56°) human serum (□– – –□), respectively. After the adherence phase, media were removed by washing, and cells were incubated in IgG-free medium prior to onset of the phagocytosis test. (a) E uptake by monocytes was assayed as a function of IgG antibodies per target E. (b) Monocytes subjected to the same treatment were tested for inhibition of erythrophagocytosis by varying doses of fluid phase IgG admixed to optimally opsonized E.

the subsequent 18 hr culture only (data not shown). In these experiments, the ratio of IgG either to serum proteins or to albumin was in the physiological range.

Modulation of FcR-dependent phagocytosis depends on a functional Fc portion of IgG

Monocytes were incubated in the presence of various types of IgG during the adherence phase, then washed and tested in the absence of IgG for their capacity to ingest opsonized E. Some of these IgG preparations were known to have a reduced capacity to bind to monocyte FcR (Jungi *et al.*, 1986a, 1986b), while others were functionally unimpaired. IgG capable of binding with high affinity to human monocyte FcR, e.g. rabbit IgG,

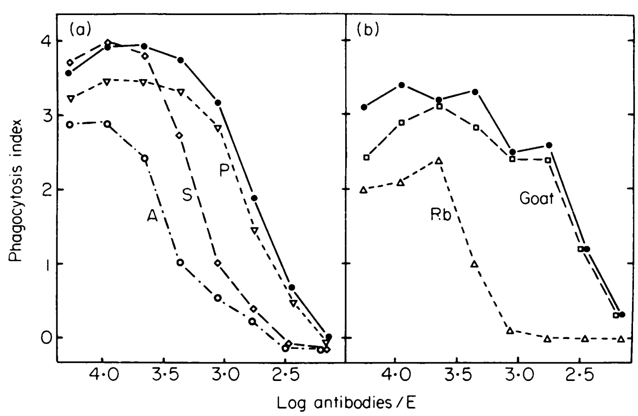

Figure 2. Influence of various types of IgG on the Fc receptor-mediated phagocytosis by monocytes. During adherence phase, monocytes were kept either in medium containing 0·5% (w/v) BSA and 0·25 mg/ml IgG (open symbols) or in medium containing BSA only (●). After washing the wells with IgG-free medium, cells were tested for E uptake as a function of IgG antibodies per E. IgG preparations included rabbit IgG, goat IgG (b) and human IgG (a). The latter consisted of intact, acid-treated IgG-IV (A), of IgG subjected to limited sulfitolysis (S), or of pepsin-treated F(ab′)₂ (P), respectively.

exhibited significant modulation of FcR-mediated phagocytosis (Fig. 2b). In contrast, goat IgG (Fig. 2b) and bovine IgG (not shown) failed to influence Fc receptor-mediated phagocytosis. Likewise, human IgG deprived of the Fc portion by pepsin treatment and IgG subjected to limited sulfitolysis had a reduced effect on monocyte phagocytosis (Fig. 2a). Thus, the capacity of IgG to modulate FcR-mediated phagocytosis was paralleled by the capacity to interact with monocyte FcR.

Monomeric IgG modulates FcR-mediated phagocytosis of monocytes

Human IgG preparations with varying proportions of dimeric and polymeric IgG were included in the medium during the monocyte adherence phase for testing their capacity to modulate phagocytosis. Heat-aggregated standard gammaglobulin was more powerful in FcR modulation than preparations poor in IgG aggregates (Fig. 3). Nevertheless, an IgG1 preparation containing no detectable aggregates and 4·3% dimers had almost the same effect on monocyte phagocytosis as IgG-IV which contained 0·5% dimeric and 1·8% polymeric IgG and ion exchange chromatography-purified IgG containing 3% dimers and 1·5% polymers. This suggested that IgG in monomeric form is able to modulate FcR-dependent phagocytosis.

Modulation of FcR-dependent phagocytosis is mediated by surface-adherent IgG

Monocytes preincubated with various IgG preparations during adherence or in a short-term suspension culture, then incubated for 30 min in the absence of IgG, were compared with respect to FcR-dependent phagocytosis. Figure 4 shows that exposure of monocytes to either monomeric or aggregated IgG in suspension had a moderate influence on FcR-dependent phagocytosis, while adhering monocytes were markedly influenced by monomeric IgG and even more so by heat-aggregated IgG.

In another set of experiments, monocytes were allowed to adhere to microtitre wells that had been precoated with medium containing varying amounts of IgG-IV, and monocytes adher-

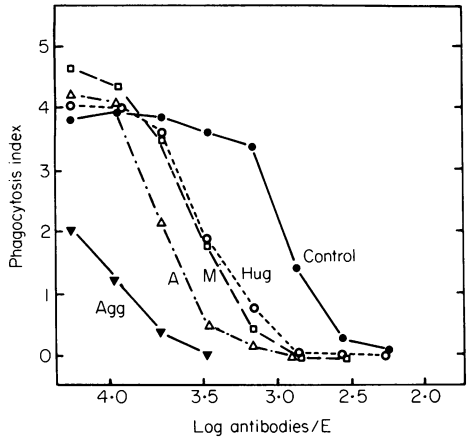

Figure 3. Influence of monomeric and aggregated IgG on the FcR-mediated phagocytosis by monocytes. During the adherence phase, monocytes were incubated in medium containing 0·5% (w/v) HSA only (●) or HA and 0·25 mg/ml IgG (▼, △, □, ○). IgG preparations included IgG-IM aggregated by heating (10 min at 62°) (Agg) and three preparations depleted from aggregates: acid-treated IgG-IV (A) contained 0·5% dimers and 1·8% polymers; monomer-enriched IgG (M) contained 3% dimers and 1·5% polymers, a monomeric fraction of IgG1 myeloma protein (Hug) contained 4·3% dimers and no detectable aggregates. The phagocytosis assay was performed as described in Fig. 2.

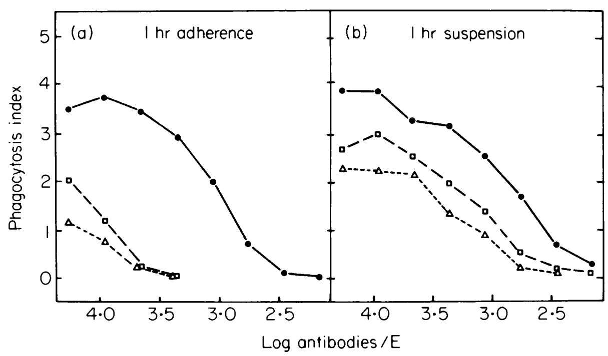

Figure 4. FcR-mediated phagocytosis of monocytes subjected to IgG either in suspension (b) or during their adherence to polystyrene (a). Mononuclear cells resuspended in medium containing 0·5% BSA alone (●), or BSA and 0·25 mg/ml monomeric IgG (□), or BSA and 0·25 mg/ml heat-aggregated IgG (△) were either allowed to adhere to microtitre plate wells or kept in suspension in Minisorp tubes for 60 min. They were washed and incubated in IgG-free medium for 30 min. During this time, suspended cells were also allowed to adhere to microtitre plate wells. Thereafter, erythrophagocytosis was assayed.

ing to such surfaces were tested for FcR-dependent phagocytosis. The degree of FcR modulation was dependent on the IgG concentration admixed to the precoating medium, provided that the concentration of non-IgG proteins (FCS or albumin) was kept constant (not shown).

Quantification of surface-bound IgG and assessment of its biological effect

The moiety of IgG adsorbing to polystyrene was determined

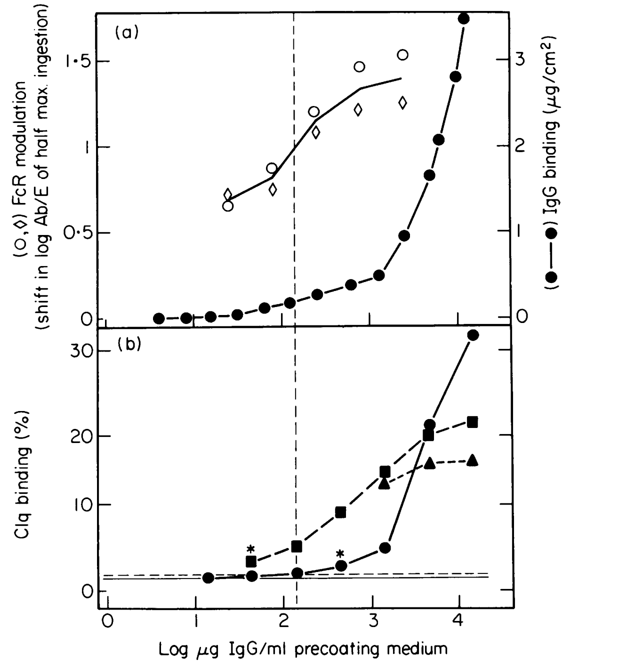

Figure 5. (a) The binding of monomer-enriched IgG diluted in MEM and 0·5% BSA to polystyrene (●) and the resulting modulation of FcR-mediated phagocytosis of monocytes adhering to the IgG-treated surface. In order to determine the degree of modulation, ingestion was quantified with E of varying opsonization degree, and the amount of antibodies required to achieve half maximal phagocytosis was calculated. This amount, in logarithmic units, was compared with that of unmodulated cells, and the difference was taken as a measure of FcR modulation. The results of two experiments (◇, ○) and their means are shown. (b) The binding of radiolabelled Clq to polystyrene pretreated with varying amounts of monomeric IgG, (●), heat-aggregated IgG-IM (■) or heat-aggregated IgG-IV (▲), respectively. The broken vertical line denotes the physiological ratio of albumin and IgG. (===) Mean + two SD of Clq fixation to IgG-free plates. (*) Lowest IgG concentration leading to significantly elevated Clq fixation ($P<0.01$).

over a broad concentration range of IgG, using ^{125}I-labelled IgG-IV. The amount of IgG bound to the microtitre wells and its effect on FcR modulation are shown in Fig. 5a. In the presence of 0·5% BSA, between 0·5% and 1% of IgG adsorbed to the plate, and less than 30 ng/cm^2 surface-bound IgG induced strong FcR modulation.

It was then tested whether monomeric IgG adsorbed to polystyrene is capable of binding complement, using ^{125}I-labelled Clq (Fig. 5b). No significantly elevated Clq binding was observed up to 200 ng/cm^2, IgG, although this IgG concentration was able to modulate phagocytosis (Fig. 5a). A small but significant elevation of Clq binding was noted up to 700 ng/cm^2 IgG, and a further increase of surface-adsorbed IgG was paralleled by an increase of Clq binding. When heat-aggregated IgG was allowed to adhere to polystyrene, as little as 50 ng/cm^2 induced significantly elevated Clq fixation.

Influence of other serum proteins on IgG absorption to polystyrene

In another set of experiments involving precoated plates, the ratio of FCS or albumin to IgG-IV was kept constant, but the absolute protein concentration during coating was varied. It

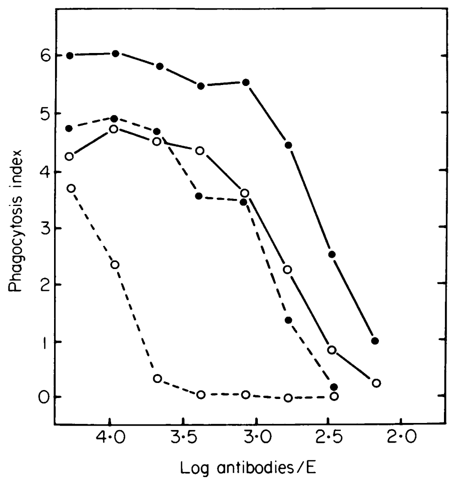

Figure 6. Protection of IgG-mediated FcR modulation of monocytes by precoating the adherence surface with FCS. Monocytes were allowed to adhere either in the absence (●) or presence (○) of IgG-IV (0·25 mg/ml) to microtitre wells. These were either untreated (– – –) or had been pretreated with FCS (20% in medium) for 4 hr at 37° (——). Adherent monocytes were washed, incubated in IgG-free medium and assayed for erythrophagocytosis. IgG-mediated FcR modulation, as expressed in a shift of the dose–response curve to the left when IgG was present, was smaller in plates precoated with FCS.

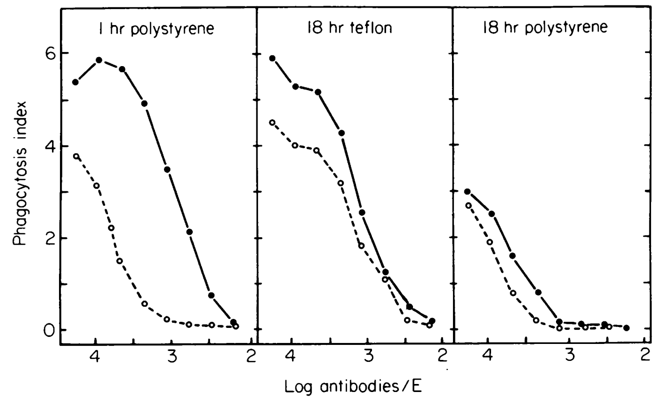

Figure 7. The influence of IgG and of prolonged contact with polystyrene on the capacity of monocytes to ingest IgG-sensitized erythrocytes. Mononuclear cells suspended either in medium containing 10% FCS alone (●), or FCS and 0·25 mg/ml IgG (○), were subjected to one of three treatments: (a) monocytes were allowed to adhere to microtitre plate wells for 60 min; (b) mononuclear cells were cultured overnight in teflon bags, harvested and washed by centrifugation; (c) mononuclear cells were cultured overnight in polystyrene tissue culture flasks, then removed from the surface by chilling and vortexing and washed by centrifugation. All cells were incubated in IgG-free medium prior to the phagocytosis test. Viability was 95% in all groups.

was found that the adherence of monocytes to plates coated with high protein concentrations was significantly diminished, resulting in the selection of strongly adherent monocytes to be assayed. Since there was a clear-cut negative correlation between the percentage of adherent monocytes and the phagocytic activity (data not shown), FcR modulation could not be unequivocally distinguished from the selection of monocyte subpopulations. However, for each concentration of non-IgG protein tested, a physiological ratio of IgG and non-IgG protein induced significant modulation of FcR-mediated phagocytosis.

Further experiments showed that precoating polystyrene first with FCS protected monocytes from the modulatory activity of IgG admixed to these cells during the adherence phase (Fig. 6).

FcR-dependent and FcR-independent modulation of FcR-mediated phagocytosis

Fresh monocyte were divided into three portions. One was tested immediately for modulation of FcR-mediated function by IgG during the adherence phase. A second portion was cultured overnight in polystyrene flasks, either in the presence or absence of IgG. A third portion was kept overnight in hydrophobic bags, either in the presence or absence of IgG. At Day 1, cells cultured overnight were harvested, washed and also subjected to the determination of FcR-mediated phagocytosis. A functional impairment with respect to FcR-mediated phagocytosis was observed when cells were cultured overnight on polystyrene in the absence of IgG, while Day 1 cells from hydrophobic containers behaved similarly to fresh monocytes (Fig. 7). A reduction of FcR-mediated phagocytosis was also manifested when cells were exposed to IgG, and a 1-hr exposure sufficed to mediate the latter impairment.

DISCUSSION

In the past, considerable interest was devoted to a modulation of Fc receptor-mediated functions by immune complexes. IgG-containing immune complexes and IgG aggregates were shown to stimulate the respiratory burst of phagocytes (Connel *et al.*, 1980; Johnston, Lehmeyer & Guthrie, 1976; Rush & Keown, 1984; Starkebaum *et al.*, 1981), to promote the release of lysosomal enzymes (Ragsdale & Arend, 1979), and to induce a state of refractoriness with respect to FcR-mediated binding, phagocytosis and cytolysis (Douglas, 1976; Rabinovitch, Manejias & Nussenzweig, 1975). The induction of refractoriness has been analysed in more detail by several groups (Kurlander, 1980; Michl *et al.*, 1979, 1983; Ragsdale & Arend, 1980). Using human monocytes, it was found that surface-adherent immune complexes impaired the FcR-mediated rosette formation and phagocytosis (Douglas, 1976, Ragsdale & Arend, 1980). The impairment was mediated by cyclic AMP and did not depend on a functioning motile apparatus. Similar findings were made with resident and elicited murine peritoneal macrophages adherent either to glass slides (Rabinovitch *et al.*, 1975) or to poly-L-lysine-coated polystyrene surfaces (Michl *et al.*, 1979, 1983) and treated with immune complexes. Following the interaction of macrophages with surface-adherent immune complexes, Fc receptors disappeared from the apical part of the cell, suggesting that the free receptors moved to the surface-attached side engaged in receptor–ligand binding. The disappearance of IgG binding sites was specific in so far as binding sites to the C3b fragment were not co-modulated (Michl *et al.*, 1979). However, later studies showed a co-modulation of Fc receptors engaged in immune complex binding and unrelated surface receptors (Sung, Nelson & Silverstein, 1985; Sung, 1985; Jack & Fearon, 1984). Thus, it is unclear at present whether the interaction of immune complexes with phagocytes ensues the redistribution and/or functional inactivation of the specific binding sites involved exclusively, or whether it leads to a more general paralysis of cellular functions.

The present report addressed another aspect of Fc receptor modulation, in that it was demonstrated that monomeric IgG also is able to promote FcR modulation in human monocytes. Two tests for the quantification of Fc receptor function have been used. First, erythrophagocytosis was quantified over a broad range of antibody densities on target erythrocytes, an assay providing reproducible dose–response curves with fresh unmodulated monocytes; when modulation occurs, higher antibody concentrations on target cells are required for promoting their ingestion. Second, the susceptibility to inhibition of Fc-mediated phagocytosis by fluid-phase IgG was quantified by measuring the ID_{50} of IgG in assays with optimally opsonized E; modulation of FcR function was associated with a decrease in ID_{50}. Using these quantitative assays, we could show that Fc receptor modulation requires a functional Fc portion and adsorption of IgG to a surface onto which monocytes adhere. However, it does not require aggregation of IgG or immune complex formation. The amount of IgG required to promote significant modulation was much smaller than that previously reported (Kurlander, 1980). This difference may be related to our use of both optimally and suboptimally sensitized E, whereas Kurlander used optimally sensitized cells only. Moreover, we found that the degree of FcR modulation rather depends on the proportion of IgG to other proteins than on the absolute concentration of IgG, and physiologial ratios of IgG to serum proteins suffice to promote significant modulation. On the other hand, our studies confirm that of Kurlander (1980) by showing that the function of monocytes is altered little when these are exposed to monomeric IgG in suspension.

Apart from the requirement for a functional Fc portion, the essential characteristics of IgG for mediating FcR modulation have not been identified. Several possibilities have to be considered. A trivial one would be a subtle structural alteration of IgG (denaturation?) induced by the artificial surface. Although IgG is susceptible to surface-induced denaturation (Miekka & Gozze, 1975), this possibility is remote since the concentration of serum and/or albumin was high enough in all our experiments to prevent such effects. Recent studies of Neppert, Marquard & Mueller-Eckhardt (1985) suggested that exposure of monocytes to antibodies directed against certain transplanation antigens causes inhibition of FcR-mediated phagocytosis. Their findings are reminiscent of Kurlander's observation (1980) that exposure of monocytes to specific antibodies induced the temporary loss of FcR function. The question arises as to whether the FcR modulation described here is mediated by a similar mechanism. However, several arguments militate against a role of specific antibodies. Firstly, polyspecific monomeric IgG had the same modulating power as an IgG1 myeloma protein (Fig. 3); secondly, fresh autologous and heterologous human sera also had similar activity; thirdly, the FcR function of monocytes was modulated by IgG preabsorbed with large numbers of autologous leucocytes (G. von Below and T. W. Jungi, unpublished observations). A further possibility to consider is that antibodies against serum components (BSA?) may form immune complexes with antigen, which then induce FcR modulation. Again, this would not explain the modulating effect of IgG1 myeloma protein. Moreover, modulation could be induced with human IgG in medium containing highly purified HSA (Fig. 3). Finally, precoating of surfaces with FCS protects monocytes from FcR modulation (Fig. 6). If anti-FCS activity were the active principle, then an enhancement of modulation by FCS-coated dishes would be expected rather than a reduction. Another possibility is that monomeric IgG undergoes allosteric alterations in the Fc portion when binding either antigen or polystyrene, and that this alteration exposes sites reactive with monocyte Fc receptors. Although our data would be compatible with this view, a number of arguments have been raised against the requirement of allosteric alterations for reactivity of IgG with Fc receptors (Phillips-Quagliata *et al.*, 1971), the most important one being that monomeric IgG readily inhibits Fc receptor-mediated phagocytosis, even in a cell suspension system (T. W. Jungi, unpublished observations). More probable is the possibility that IgG adsorbed to polystyrene acts like a multimeric complex in which positive co-operative effects lead to enhanced binding of receptors to the ligand (Leslie, 1985, Phillips-Quagliata *et al.*, 1971). This binding could, in addition, promote receptor cross-linking, which in turn might trigger cellular functions including receptor re-distribution (modulation).

Attempts to quantify the relationship between IgG density on polystyrene surfaces and functional modulation showed that both the amount of IgG adsorbed and the extent of functional impairment depend on the medium composition, and experiments with a two-step incubation (first albumin, then IgG) suggested that there is a competition between IgG and other serum components with respect to surface adherence. When converting the amount of modulatory IgG to surface density, suboptimal modulation is already observed with 30 ng IgG/cm^2, which would correspond to one IgG molecule per 830 nm^2 if IgG formed a monolayer; a 100-fold higher surface density, which already provides optimal modulation, would be close to complete saturation, given the cross-sectional area of an IgG molecule of 60 nm^2. Since similar amounts of albumin are simultaneously bound to the surface, it appears that the surface-bound proteins do not correspond to monolayer arrangement. Steric reasons may in addition prevent a certain portion of IgG from interacting with monocytes. Nevertheless, using identical conditions for binding assays and for functional measurements, the IgG density on polystyrene required to promote increased Clq fixation was >10-fold higher than that required for FcR modulation. IgG Fc portions must be closely adjacent in order to bind the multimeric Clq ligand, while at physiological temperature Fc receptors are able to move in the cell membrane towards appropriate Fc ligands (Michl *et al.*, 1983, Leslie, 1985). In addition, monomeric Clq binding sites have a much lower binding affinity to the IgG Fc portion (Hugh-Jones & Gardner, 1979) than have monocyte Fc receptors (Alexander *et al.*, 1978; Jungi & Hafner, 1986). In any case, modulation of monocyte Fc receptor-mediated functions may be regarded as a highly sensitive indicator for surface-immobilized IgG.

FcR modulation occurred regardless of whether IgG was used for precoating polystyrene, whether it was present during the adherence phase, or whether cells were exposed to it after they had adhered. This finding suggests that adherent cells are in a dynamic state of continuing interaction with new IgG-coated surface areas in their proximity. Monocytes or macrophages that have undergone FcR modulation may recover from this impairment. This requires protein synthesis (Kurlander, 1980), and the time required for restoration of the function varies from system to system. Human monocytes cultured overnight in the presence of IgG-containing serum and then transferred to teflon bags require at least 6 days until FcR-mediated phagocytosis and rosette formation have fully regenerated, regardless of whether IgG is present or absent from the teflon bag culture (Jungi & Hafner, 1986). Interestingly, recovery can occur even in adherence cultures in the continued presence of IgG (Newman, Musson & Henson, 1980). When recovered macrophages kept for 8 days *in vitro* were exposed to IgG during a second adherence phase, their FcR function was modulated in the same way as that of fresh monocytes (T. W. Jungi, unpublished observations). A full understanding of the regeneration mechanism would require the use of cell culture systems with media of defined composition.

A finding not previously reported was our observation that monocytes exposed to polystyrene in an overnight culture in the absence of IgG were functionally impaired when compared with cells kept in teflon bags during the same period of time. It was concluded that functional impairment can be achieved in two ways: in a receptor-specific way occurring within less than 1 hr, and in a non-specific manner, mediated by certain surfaces, in a more protracted way.

In the light of this profound functional impairment by relatively small amounts of a physiological tissue fluid component, the question arises as to whether this functional inhibition has an *in vivo* correlate. Receptor modulation, as described here, has profound influences on the capacity to bind and ingest IgG-opsonized particles, and this could apply to bacteria infecting artificial implants. Prosthetic material could adsorb IgG in amounts too small to induce complement fixation, but large enough to promote FcR modulation in phagocytes adhering to the implant. Even though the functional defect would be overcome with time, a subtle functional defect in the early phase of an infection could be determining for their fate. Not only was the number of antibodies required for mediating ingestion significantly increased, but the inhibition exerted by IgG in the tissue fluids would be enhanced. Although our present *in vitro* model, using polystyrene as an anchoring surface for phagocytes, is relatively non-physiological, it seems worthwhile to investigate the function of phagocytes exposed to IgG-coated materials used in surgery. Another area in which the present findings might be relevant is haemodialysis and transfusion medicine. Thus, the function of phagocytes following exposure to leukapheresis, plasmapheresis or haemodialysis equipment has not been sufficiently analysed in this regard.

ACKNOWLEDGMENTS

This work was supported by the Central Laboratory of the Swiss Red Cross Blood Transfusion Service, the Stanley Thomas Johnson Foundation, and the Swiss National Science Fund, Grant No. 3.827.0·83. The excellent technical assistance by Mrs M. Brčić is gratefully acknowledged. The critical reading of the manuscript by Professor A. Morell is appreciated.

REFERENCES

ALEXANDER M.D., ANDREWS J.A., LESLIE R.G.Q. & WOOD N.J (1978) The binding of human and guinea-pig IgG subclasses to homologous macrophage and monocyte Fc receptors. *Immunology*, **35**, 115.

BØYUM A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Lab. Invest.* **21**, Suppl. 97, 77.

CONNEL P.A., SEEHRA M.S., LESLIE R.G.Q. & REEVES W.G. (1980) Chemiluminescence of guinea pig peritoneal macrophages stimulated by immune precipitates and soluble immune complexes. *Eur. J. Immunol.* **19**, 966.

DOUGLAS S.D. (1976) Human monocyte spreading *in vitro* — inducers and effects on Fc and C3 receptors. *Cell. Immunol.* **21**, 344.

HUGHES-JONES N.C. & GARDNER B. (1979) Reaction between the isolated globular sub-units of the complement component Clq and IgG-complexes. *Molec. Immunol.* **16**, 697.

JACK R.M. & FEARON D.T. (1984) Altered surface distribution of both C3b receptors and Fc receptors on neutrophils induced by anti-C3b receptor or aggregated IgG. *J. Immunol.* **132**, 3028.

JOHNSTON R.B., LEHMEYER J.E. & GUTHRIE L.A. (1976) Generation of superoxide anion and chemiluminescence by human monocytes during phagocytosis and on contact with surface-bound immunoglobulin G. *J. exp. Med.* **143**, 1551.

JUNGI T.W. (1985) A rapid and sensitive method allowing photometric determination of erythrophagocytosis by mononuclear phagocytes. *J. Immunol. Meth.* **82**, 141.

JUNGI T.W. & BARANDUN S. (1985) Estimation of the degree of opsonization of homologous erythrocytes by IgG for intravenous and intramuscular use. *Vox Sang.* **49**, 9.

JUNGI T.W., EIHOLZER J., LERCH P.G. & BARANDUN S. (1986a) The capacity of various types of immunoglobulin for intravenous use to interact with Fc receptors of human monocytes and macrophages. *Blut*, **53**, 321.

JUNGI T.W. & HAFNER S. (1986) Quantitative assessment of Fc receptor expression and function during *in vitro* differentiation of human monocytes to macrophages. *Immunology*, **58**, 131.

JUNGI T.W., SANTER M., LERCH P.G. & BARANDUN S. (1986b) Effect of various treatments of gamma-globulin (IgG) for achieving intravenous tolerance on the capacity to interact with human monocyte Fc receptors. *Vox Sang.* **51**, 18.

KABAT E.A. & MAYER M.M. (1961) *Experimental Immunochemistry*, 2nd edn. Thomas, Springfield.

KOLB W.P., KOLB L.M. & PODACK E.P. (1979) Clq isolation from human serum in high yield by affinity chromatography and development of a highly sensitive hemolytic assay. *J. Immunol.* **122**, 2103.

KURLANDER R.J. (1980) Reversible and irreversible loss of Fc receptor function of human monocytes as a consequence of interaction with immunoglobulin. *J. clin. Invest.* **66**, 773.

LESLIE R.G.Q. (1985) Complex aggregation; a critical event in macrophage handling of soluble immune complexes. *Immunol. Today*, **6**, 183.

MICHL J., PIECZONKA M.M., UNKELESS J.C. & SILVERSTEIN S.C. (1979) Effects of immobilized immune complexes on Fc-γ and complement-receptor function in resident and thioglycollate-elicited mouse peritoneal macrophages. *J. exp. Med.* **150**, 607.

MICHL J., UNKELESS J.C., PIECZONKA M.M. & SILVERSTEIN S.C. (1983) Modulation of Fc receptors of mononuclear phagocytes by immobilized antigen–antibody complexes. Quantitative analysis of the relationship between ligand number and Fc receptor response. *J. exp. Med.* **157**, 1746.

MIEKKA S.I. & GOZZE I. (1975) Anticomplementary activity of human immunoglobulin G. *Vox Sang.* **29**, 101.

NAKAGAWARA W. & NATHAN C.F. (1983) A simple method for counting adherent cells: application to cultured human monocytes, macrophages and multinucleated giant cells. *J. immunol. Meth.* **56**, 261.

NEPPERT J., MARQUARD F. & MUELLER-ECKHARDT C. (1985) Murine monoclonal antibodies and human alloantisera specific for HLA inhibit monocyte phagocytosis of anti-D-sensitized human red blood cells. *Eur. J. Immunol.* **15**, 559.

NEWMAN S.L., MUSSON R.A. & HENSON P.M. (1980) Development of functional complement receptors during *in vitro* maturation of human monocytes into macrophages. *J. Immunol.* **125**, 2236.

PHILLIPS-QUAGLIATA J.M., LEVINE B.B., QUAGLIATA F. & UHR J.W. (1971) Mechanisms underlying binding of immune complexes to macrophages. *J. exp. Med.* **133**, 589.

RABINOVITCH M., MANEJIAS R.E. & NUSSENZWEIG V. (1975) Selective phagocytic paralysis induced by immobilized immune complexes. *J. exp. Med.* **149,** 827.

RAGSDALE C.G. & AREND W.P. (1979) Neutral protease secretion by human monocytes. Effect of surface-bound immune complexes. *J. exp. Med.* **149,** 954.

RAGSDALE C.G. & AREND W.P. (1980) Loss of Fc-receptor activity after culture of human monocytes on surface-bound immune complexes. *J. exp. Med.* **151,** 32.

RUSH D.N. & KEOWN P.A. (1984) Human monocyte chemiluminescence triggered by IgG aggregates. Requirement of phospholipase activation and modulation by Fc-receptor ligands. *Cell. Immunol.* **87,** 252.

SILVERSTEIN S.C., STEINMAN R.M. & COHN Z.A. (1977) Endocytosis. *Ann. Rev. Biochem.* **46,** 669.

SONODA S. & SCHLAMOWITZ M. (1970) Studies of ^{125}I trace labeling of immunoglobulin G by chloramine T. *Immunochemistry*, **7,** 885.

SPAETH P.J., CORVETTA A., NYDEGGER U.E., MONTRONI M. & BUETLER R. (1983) An extended Clq-binding assay using lactoperoxidase- and chloramine-T-iodinated Clq. *Scand. J. Immunol.* **18,** 319.

STARKEBAUM G., STEVENS D.L., HENRY C. & GAVIN S.E. (1981) Stimulation of human neutrophil chemiluminescence by soluble immune complexes and antibodies to neutrophils. *J. Lab. clin. Med.* **98,** 280.

SUNG S.-S.J. (1985) Phagocytosis by mouse peritoneal macrophages plated on monoclonal antibody-coated immune complex-substrates: effects of complexes of different IgG subclasses on Fc receptor functions. *J. Immunol.* **135,** 1981.

SUNG S.-S.J., NELSON R.S. & SILVERSTEIN S.C. (1985) Mouse macrophages plated on mannan- and horseradish peroxidase-coated substrates lose the ability to phagocytose by their Fc receptors. *J. Immunol.* **134,** 3712.